

CLAIMS

1. A composition for producing an antibody, comprising: (1) a polypeptide comprising the amino acid sequence of SEQ ID NO: 2; (2) a polypeptide having an amino acid sequence mutated from the amino acid sequence of SEQ ID NO: 2 by the substitution, deletion, addition, and/or insertion of one or more amino acid residues and inducing the production of an antibody specific to the polypeptide comprising the amino acid sequence of SEQ ID NO: 2; or (3) a polypeptide fragment having a partial sequence of the polypeptide of (1) or (2) and inducing the production of an antibody specific to the polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
- 10 2. The composition according to claim 1, wherein the antibody is an antibody for detecting a human cancer cell.
3. The composition according to claim 1, comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
4. The composition according to claim 1, comprising a polypeptide having a partial sequence of the amino acid sequence of SEQ ID NO: 2 and inducing the production of an antibody specific to the polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
- 15 5. The composition according to claim 1, comprising a polypeptide fragment having a partial sequence of the amino acid sequence of SEQ ID NO: 2 and inducing the production of an antibody specific to the polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
- 20 6. A method for producing an antibody specific to a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, comprising administering a composition according to claim 1 to a mammal.
- 25 7. An antibody specific to a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.
8. The antibody according to claim 7, wherein the antibody is a human/mouse chimeric antibody, a humanized antibody, or a human antibody.
9. The antibody according to claim 7, wherein the antibody is a polyclonal antibody or 30 a monoclonal antibody.
10. A diagnostic method for cancer, comprising bringing an antibody according to claim 7 into contact with a biological sample.
11. A diagnostic kit for cancer, comprising an antibody according to claim 7.

12. A pharmaceutical composition for cancer therapy, comprising an antibody according to claim 7.
13. The pharmaceutical composition for cancer therapy according to claim 12, wherein the antibody is a human/mouse chimeric antibody, a humanized antibody, or a human antibody.
14. The pharmaceutical composition for cancer therapy according to claim 12, wherein the antibody is a polyclonal antibody or a monoclonal antibody.
15. The pharmaceutical composition for cancer therapy according to claim 12, further comprising an appropriate carrier.
- 10 16. A diagnostic method for cancer, characterized by detecting a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof.
17. The diagnostic method for cancer according to claim 16, wherein the polynucleotide or the fragment thereof is detected by polymerase chain reaction (PCR) or real-time quantitative polymerase chain reaction.
- 15 18. The diagnostic method for cancer according to claim 17, wherein the detection is performed by PCR using a sense strand fragment corresponding to the nucleotide sequence of SEQ ID NO: 1 as a forward primer and an antisense strand fragment corresponding to the nucleotide sequence of SEQ ID NO: 1 as a reverse primer.
19. The diagnostic method for cancer according to claim 18, wherein the forward primer has 14 to 60 bases in length and the reverse primer has 14 to 60 bases in length.
- 20 20. The diagnostic method for cancer according to claim 18, wherein the diagnostic method uses the following forward and reverse primers:
the forward primer of 5'- GGAGCAGGAATTGGGGTCAC -3' (SEQ ID NO: 5);
and
25 the reverse primer of 5'- TTGCTGTCCCATCCGGTGAG -3' (SEQ ID NO: 6).
21. The diagnostic method for cancer according to claim 17, wherein the detection is performed by real-time quantitative polymerase chain reaction using a sense strand fragment corresponding to the nucleotide sequence of SEQ ID NO: 1 as a forward primer and an antisense strand fragment corresponding to the nucleotide sequence of SEQ ID NO: 1 as a reverse primer.
- 30 22. The diagnostic method for cancer according to claim 21, wherein the diagnostic method uses the following forward and reverse primers and TaqMan probe:
the forward primer of 5'- CCACTGTAGGCGCCCTAAGTT -3' (SEQ ID NO: 7);

the reverse primer of 5'- AAGAATGACCGGTGCAAGGA -3' (SEQ ID NO: 8);
and

the TaqMan probe of 5'- AAGGGCATCCCCCTGAGTCTTGGAA -3' (SEQ ID NO: 9).

- 5 23. siRNA corresponding to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof.
24. The siRNA according to claim 23, corresponding to a polynucleotide comprising a nucleotide sequence at positions from 71 to 1615 of SEQ ID NO: 1 or a fragment thereof.
25. The siRNA according to claim 23, wherein the siRNA has a nucleotide sequence
10 from 8 to 30 bp in length.
26. The siRNA according to claim 23, wherein the siRNA consists of a nucleotide sequence selected from the group consisting of the following nucleotide sequences of SEQ ID NOs: 10 to 14:

5'-GCGTGGCTTCAGCATGGAATTCAAGAGATTCCATGCTGAAGGCCACGCTTTT
15 TGGAAA-3' (SEQ ID NO: 10);
5'-GGGCTTCGAACAACAATTCAAGAGATATTGTTGTCGAAAGCCCTTTT
TGGAAA-3' (SEQ ID NO: 11);
5'-GTTATGAGAACAGTCTGACAAGTTCAAGAGACTTGTCAGACTTCTCATAATT
TTGGAAA-3' (SEQ ID NO: 12);
20 5'-GATTCTTGGCTAAATCCCATTCAAGAGATGGGATTAGCCAAGAACATTTTT
TGGAAA-3' (SEQ ID NO: 13); and
5'-GGACATTGAACAACAGCATTCAAGAGATGCTGTTCAAATGTCCTTTT
TGGAAA-3' (SEQ ID NO: 14).
27. A pharmaceutical composition for cancer therapy, comprising: siRNA
25 corresponding to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof; and siRNA corresponding to a polynucleotide comprising a nucleotide sequence at positions from 71 to 1615 of SEQ ID NO: 1 or a fragment thereof.
28. A pharmaceutical composition for cancer therapy, comprising a cell transformed
30 with siRNA corresponding to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof; and siRNA corresponding to a polynucleotide comprising a nucleotide sequence at positions from 71 to 1615 of SEQ ID NO: 1 or a fragment thereof.
29. The pharmaceutical composition for cancer therapy according to claim 28, wherein
the cell to be transformed is a cell taken out of a patient to be treated.

30. A method for producing a cell for cancer therapy, comprising preparing a human cell and transforming the cell with siRNA corresponding to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 or a fragment thereof; and siRNA corresponding to a polynucleotide comprising a nucleotide sequence at positions from 71 to 1615 of SEQ ID NO: 1 or a fragment thereof.
31. The method for producing a cell for cancer therapy according to claim 30, wherein the human cell is a cell taken out of a patient to be treated.
32. A screening method for a cancer cell growth inhibitor targeted for a Nox1 gene, comprising: transfecting a cell having a mutant Ras gene with a Nox1 gene; bringing the transformed cell into contact with a substance to be screened; and detecting the expression of the Nox1 gene and the inactivation of Nox1 activity.
33. The screening method according to claim 32, comprising culturing the transformed cell together with the substance to be screened.
34. The screening method according to claim 32, wherein the expression of the Nox1 gene is detected by detecting mRNA by real-time quantitative polymerase chain reaction or detecting a polypeptide or a peptide fragment thereof coded by the Nox 1 gene with an antibody.
35. The screening method according to claim 32, wherein the expression of the Nox1 gene is detected by observing morphological changes in the transformed cell.
36. The screening method according to claim 32, wherein the cell having a mutant Ras gene is an H-Ras-NIH3T3 cell or a K-Ras-NRK cell.
37. The screening method according to claim 32, wherein the transfection of the Nox1 gene is performed using pEGFP-C1 (K-Ras-NRK/GFP) or pEGFP-C1-Nox1 (K-Ras-NRK/GFP-Nox1).
38. A cell having a mutant Ras gene, which is transfected with a Nox1 gene.

ABSTRACT

The present invention provides a diagnostic method for cancer, a screening method for a cancer growth inhibitor, and a pharmaceutical composition used in cancer therapy using a Nox1 gene associated with a mutant Ras oncogene. More specifically, the present

- 5 invention relates to: a composition for producing an antibody, comprising a polypeptide coded for a Nox1 gene, a homologue thereof, and their peptide fragments; an antibody against the polypeptide coded for a Nox1 gene; and a method for detecting the antibody or Nox1-expressing mRNA.